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Plasmid-Mediated Resistance to Expanded-Spectrum Cephalosporins among *Enterobacter aerogenes* Strains

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Resistance to expanded-spectrum cephalosporins commonly develops in *Enterobacter aerogenes* during therapy due to selection of mutants producing high levels of the chromosomal Bush group 1 β-lactamase. Recently, resistant strains producing plasmid-mediated extended-spectrum β-lactamases (ESBLs) have been isolated as well. A study was designed to investigate ESBL production among 31 clinical isolates of *E. aerogenes* from Richmond, Va., with decreased susceptibility to expanded-spectrum cephalosporins and a positive double-disk potentiation test. Antibiotic susceptibility was determined by standard disk diffusion and agar dilution procedures. β-Lactamases were investigated by an isoelectric focusing overlay technique which simultaneously determined isoelectric points (pIs) and substrate or inhibitor profiles. Decreased susceptibility to cefotaxime, ceftazidime, and aztreonam (MIC range, 1 to 64 μg/ml) was detected and associated with resistance to gentamicin and trimethoprim-sulfamethoxazole. All strains produced an inducible Bush group 1 β-lactamase (pI 8.3). Twenty-nine of the 31 isolates also produced an enzyme similar to SHV-4 (pI 7.8), while 1 isolate each produced an enzyme similar to SHV-3 (pI 6.9) and to SHV-5 (pI 8.2). The three different SHV-derived ESBLs were transferred by transconjugation to *Escherichia coli* C600N and amplified by PCR. Plasmid profiles of the clinical isolates showed a variety of different large plasmids. Because of the linkage of resistance to aminoglycosides and trimethoprim-sulfamethoxazole with ESBL production, it is possible that the usage of these drugs was responsible for selecting plasmid-mediated resistance to extended-spectrum cephalosporins in *E. aerogenes*. Furthermore, it is important that strains such as these be recognized, because they can be responsible for institutional spread of resistance genes.

Enterobacter species are becoming increasingly important nosocomial pathogens (29). In the most recent National Nosocomial Infections Study data published, *Enterobacter* is the third-most-common pathogen recovered from the respiratory tract (16). Data from isolates recovered from intensive care units revealed that this organism was also the fourth-most-common pathogen recovered from surgical wounds, the fifth-most-common pathogen recovered from the urinary tract, and the fifth-most-common pathogen recovered from blood (16). Risk factors for nosocomial *Enterobacter* infection include the prior use of antimicrobial agents, a prolonged hospital stay, a serious underlying illness, immunosuppression, and the presence of a foreign device (29).

Resistance to expanded-spectrum cephalosporins, broad-spectrum penicillins, and aztreonam usually emerges in *Enterobacter* spp. due to a mutation in a chromosomal gene, *ampD*, that normally prevents high-level expression of this organism's chromosomal β-lactamase (27). This mutation results in high-level production of the chromosomal Bush group 1 β-lactamase (5), rendering the organisms resistant to all β-lactam antibiotics except the carbapenems and cefepime. Such *ampD* mutants have often been referred to as stably derepressed mutants (27). The increase in the prevalence of resistance to expanded-spectrum cephalosporins among species of *Enterobacter* has been associated with the increased use of the more newly developed cephalosporins (6, 10). It is now well established that stably derepressed mutants of *Enterobacter* spp. may be selected during therapy with a number of β-lactam drugs, especially expanded-spectrum cephalosporins such as ceftazidime, cefotaxime, and ceftriaxone (6, 7, 10, 17, 31).

Recently, a different mechanism of resistance to expanded-spectrum cephalosporins has been recognized in species of *Enterobacter*. This involves wild-type strains of *Enterobacter* acquiring plasmids encoding Bush group 2be β-lactamases, the extended-spectrum β-lactamases (ESBLs). *Enterobacter* isolates producing ESBLs have been recovered from patients in France (8, 9, 11), the United States (26), and the United Kingdom (12). These organisms have acquired large plasmids encoding a variety of ESBLs, including TEM-3, TEM-10, TEM-12, TEM-24, and TEM-26. In addition, some of these plasmids also encoded resistance to the aminoglycosides, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole (9, 11, 12). ESBL production has been far more common in *Enterobacteriaceae* lacking inducible group 1 β-lactamases, such as *Klebsiella pneumoniae* and *Escherichia coli* (14, 24). Thus, the recovery of large numbers of ESBL-producing *Enterobacter aerogenes* strains from a single hospital was quite unusual.

A number of strains of *E. aerogenes* recovered from patients at the Hunter Holmes McGuire Medical Center in Richmond, Va., displayed a resistant phenotype different from that of the derepressed mutants normally encountered in this species. This phenotype involved decreased susceptibility to ceftriaxone and high-level resistance to ceftazidime. Derepressed mutants previously isolated from this center were usually resistant to both ceftriaxone and ceftazidime, while the wild type remained sensitive to both β-lactams. Therefore, a study was

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designed to investigate and describe the possible mechanisms responsible for the resistance to the expanded-spectrum cephalosporins that was observed in *E. aerogenes* strains isolated from this hospital.

MATERIALS AND METHODS

Bacterial strains. Among all *E. aerogenes* strains ($n = 184$) recovered from clinical specimens during an 18-month period (September 1993 to March 1995), 31 (16.8%) strains showing a resistance phenotype different from that observed with the derepressed mutants normally encountered at the Hunter Holmes McGuire Medical Center were selected for this study. The strains selected were shown to be intermediate to ceftriaxone but resistant to ceftazidime by the Vitek automated susceptibility system (bioMérieux Vitek, St. Louis, Mo.). Derepressed mutants previously isolated from this center were usually resistant to both ceftriaxone and ceftazidime.

Susceptibility testing. Antibiotic susceptibilities were determined by standard disk diffusion (21) and agar dilution (22) procedures. Disks were obtained from Becton Dickinson Microbiology Systems (Cockeysville, Md.). Disk diffusion susceptibilities to the following antibiotics were determined: ampicillin, amoxicillin clavulanic acid, aztreonam, cefazolin, cefoxitin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime, imipenem, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin. Standard powders of antimicrobial agents for MIC determinations were kindly provided by the following companies: Merck (Rahway, N.J.) (cefoxitin and imipenem), Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N.J.) (cefotaxime), Glaxo Group Research Ltd. (Greenford, England) (ceftazidime), Bristol-Myers Squibb (Princeton, N.J.) (aztreonam and cefepime), and Schering-Plough (Liberty Corner, N.J.) (gentamicin). The following quality control strains were run simultaneously with the test organisms: *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 35218. Throughout this study, results were interpreted with National Committee for Clinical Laboratory Standards criteria for disk diffusion (21) and broth dilution (22).

Double-disk potentiation test. This test, described by Jarlier et al. (15) with ceftazidime, ceftriaxone, cefotaxime, and aztreonam disks, was performed on the strains to screen for possible ESBL production. This test is a modification of the disk diffusion susceptibility test in that cefotaxime, ceftriaxone, ceftazidime, and aztreonam disks are placed 30 mm from disks containing amoxicillin-clavulanic acid. A potentiation of the zones of cefotaxime, ceftriaxone, ceftazidime, or aztreonam by clavulanic acid represented a positive test and was indicative of possible ESBL production.

β-Lactamase preparation, IEF, and assays. Overnight cultures in 5 ml of Mueller-Hinton broth were diluted with 95 ml of fresh broth and incubated with shaking for 90 min at 37°C. One-fourth of the cefoxitin MIC was added for induction, while sterile medium was used in the noninduced cultures and incubated for an additional 2 h. The induction process was stopped by the addition of 1 mM 8-hydroxyquinoline solution to each culture. Cells were harvested by centrifugation at 4°C, washed with 1 M potassium-phosphate buffer (pH 7.0), suspended, and sonicated. After sonication, crude extracts were obtained by centrifugation at 6,000 rpm for 1 h. The β-lactamases in the sonic extracts were assessed for isoelectric points (pIs) and substrate and inhibitor profiles in polyacrylamide gels with overlays of 0.75 μg of cefotaxime per ml, 1,000 μM clavulanic acid, and 1,000 μM cloxacillin prior to overlay with nitrocefin agar (1, 19, 28). Cephalothin hydrolysis rates were determined by UV spectrophotometric assay (23). Inducibility of the Bush group 1 β-lactamase was inferred from the intensity of isoelectric focusing (IEF) patterns for uninduced and induced β-lactamase extracts. As controls, crude β-lactamase preparations from the following organisms possessing different SHV enzymes were evaluated simultaneously with those obtained from the *Enterobacter* strains: SHV-1 [from *E. coli* J53(R1010)], SHV-2 (from *Klebsiella ozaenae* 2180), SHV-3 [from *E. coli* J53-2(pUD18)], SHV-4 [from *E. coli* J53-2(pUD21)], and SHV-5 [from *E. coli* Cla Nal(pAFF2)].

Isolation of plasmids. The organisms were inoculated into 5 ml of Luria Bertani (LB) broth (Difco, Detroit, Mich.) and incubated for 20 h at 37°C with shaking. Cells from 1.5 ml of overnight culture were harvested by centrifugation in an Eppendorf centrifuge for 5 min. After the supernatant was decanted, the pellet was resuspended in 1% Triton X-100 in Tris-EDTA buffer for 10 min. Plasmid DNA was then isolated by the alkaline extraction method of Birnboim and Doly (3) and separated by electrophoresis in 0.8% agarose gel (Sigma) in TAE buffer (0.04 M Tris-acetate, 0.002 M EDTA [pH 8.5]). The gel was stained with ethidium bromide, and plasmid bands were visualized with UV light.

Conjugation experiments. To determine if the resistance was transferable, transconjugation experiments were performed with *E. coli* C600N (Nal^r) as the recipient (18). The filter paper mating technique with overnight incubation at 37°C was performed as described previously (25). Transconjugants were selected on LB agar (Difco) plates containing 12 μg of nalidixic acid per ml and 20 μg of ampicillin per ml.

DNA amplification by PCR. Organisms were inoculated into 5 ml of LB broth (Difco) and incubated for 20 h at 37°C with shaking. Cells from 1.5 ml of overnight culture were harvested by centrifugation at 13,000 rpm in an Eppendorf centrifuge for 5 min. After the supernatant was decanted, the pellet was resuspended in 500 μl of sterile deionized water. The cells were lysed by heating to 95°C for 10 min, and cellular debris was removed by centrifugation for 5 min at 13,000 rpm. The supernatant was used as the source of template for amplification. Oligonucleotide primers specific for SHV genes were selected from a consensus alignment sequence generated by the MacVector 4.5 (Kodak/IBI) software package from the published nucleotide sequences of SHV-1 (20), SHV-2 (13), SHV-5 (2), and SHV-7 (4). The sequences of the PCR primers used were A [5′-(CACTCAAGGATGTATTGTG)-3′] and B [5′-(TTAGCGTTGCC AGTGCTCG)-3′], which amplified a 781-bp fragment. Primer specificity controls included the TEM-1, MIR-1, and SHV-7 β-lactamase genes. PCR amplifications were carried out on a DNA Thermal Cycler 480 instrument (Perkin-Elmer Cetus, Norwalk, Conn.) with the GeneAmp DNA amplification kit containing Ampli*Taq* polymerase (Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, N.J.). The composition of the reaction mixture was as follows: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, deoxynucleoside triphosphates (0.2 mM each), and 1.2 U of Ampli*Taq* in a total volume of 49 μl. A total of 1 μl of sample lysate was added to the reaction mixture, which was centrifuged briefly before 50 μl of mineral oil was layered onto the surface. The PCR program consisted of an initial denaturation step at 96°C for 30 s followed by 24 cycles of DNA denaturation at 96°C for 30 s, primer annealing at 50°C for 15 s, and primer extension at 72°C for 2 min. After the last cycle, the products were stored at 4°C. The PCR products were analyzed by electrophoresis with 1.4% agarose gels in TAE buffer. The gels were stained with ethidium bromide, and the PCR products were visualized with UV light.

RESULTS

Bacterial strains. Twenty-four of the 31 strains from Hunter Holmes McGuire Medical Center originated from patients in two spinal cord injury wards (SCW1 and SCW2), while 3 strains were isolated from patients in the medical intensive care unit, 3 isolates were recovered from patients attending the surgical outpatient clinic, and 1 isolate was recovered from a patient in a general surgery ward. Disk diffusion susceptibility tests showed all the strains to be resistant to ampicillin, amoxicillin-clavulanate, cefazolin, cefuroxime, and trimethoprim-sulfamethoxazole and all the strains to be susceptible to ciprofloxacin. MICs of cefoxitin, cefotaxime, ceftazidime, aztreonam, cefepime, imipenem, and gentamicin are summarized in Table 1. All strains were susceptible to cefepime and imipenem but showed decreased susceptibility to cefotaxime, ceftazidime, and aztreonam. MICs of gentamicin ranged from 8 to >128 μg/ml for 25 of 37 (67%) isolates. All the strains selected for this study showed a positive double-disk test when cefotaxime and ceftriaxone disks were used.

Characteristics of β-lactamases. All the *Enterobacter* isolates possessed a Bush group 1 inducible β-lactamase with an alkaline pI of 8.3 which was sensitive to inhibition by cloxacillin but not clavulanic acid (Table 1). Additional Bush group 2be enzymes with pIs resembling SHV β-lactamases were also present in all the strains (Table 1). Three different group 2be enzymes were detected in the species of *Enterobacter* (Table 1): the majority of isolates (29 of 31) produced an enzyme with a pI of 7.8, which aligned with SHV-4. One isolate produced an enzyme with a pI of 6.8, which aligned with SHV-3, and one isolate produced an enzyme with a pI of 8.2, which aligned with SHV-5.

Plasmid profiles. A variety of different plasmids with sizes ranging from 10 to approximately 60 kb were visualized with electrophoresis (Table 2). Furthermore, eight different plasmid patterns were observed, with the number of plasmids ranging from 0 to 5 per organism (Table 2). No plasmids were visualized in three strains, including the strain which produced an enzyme resembling SHV-5 (Table 2). Three different susceptibility profiles were identified (Table 2). The majority of organisms isolated were resistant to ceftazidime, aztreonam, trimethoprim-sulfamethoxazole, and gentamicin. This antibiogram was associated with the production of β-lactamases resembling SHV-4 and SHV-5 and isolated from SCW1 and SCW2, the medical intensive care unit, the general surgical ward, and the outpatient clinic (Table 2). Eight of thirty strains

TABLE 1. MICs and characteristics of β-lactamases produced by *E. aerogenes*

No. of strains	β-Lactamase group (enzyme type)[b]	Enzyme characteristic					MIC (μg/ml) of[a]:						
		pI	CTX hydrolysis[c]	Inhibited by[d]:		Inducible[e]	FOX	CTX	CAZ	ATM	FEP	IMI	GM
				CLOX	CLAV								
1	1	8.3	No	Yes	No	Yes	>256	1	4	1	0.12	0.5	8
	2be (SHV-3)	6.9	Yes	No	Yes	No							
29	1	8.3	No	Yes	No	Yes	>256	1–2	8–32	32–64	0.12–1	0.5	1–16
	2be (SHV-4)	7.8	Yes	No	Yes	No							
1	1	8.3	No	Yes	No	Yes	>256	1–2	32	64	1	1	>128
	2be (SHV-5)	8.0	Yes	No	Yes	No							
4	1[f] (wild type)	8.3–8.8	No	Yes	No	Yes	≥64	≤1.0	≤1.0	≤0.5	≤0.12	≤0.5	≤1.0
5	1[f] (derepressed)	8.3–8.9	Yes	Yes	No	No	>256	≥32	≥64	16–64	≤1.0	≤1.0	1–128

[a] FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; FEP, cefepime; IMI, imipenem; GM, gentamicin.
[b] Based on Bush-Jacoby-Medeiros classification (5). The β-lactamase listed in parentheses is the one most similar to the group 2be enzyme produced by the *Enterobacter* strains.
[c] Hydrolysis of 0.75 μg of cefotaxime (CTX) per ml used in substrate-based IEF overlay technique (1).
[d] Inhibitors used in IEF overlay technique were clavulanic acid (CLAV; 1,000 μM) and cloxacillin (CLOX; 1,000 μM) (28).
[e] Inducible by cefoxitin.
[f] Unpublished data from the Center for Research in Anti-Infectives and Biotechnology.

showing three different plasmid profiles (a, b, and f) and producing an enzyme resembling SHV-4 isolated from SCW1 as well as from the surgical outpatient clinic were susceptible to gentamicin, while the *E. aerogenes* strain producing an enzyme resembling SHV-3 appeared susceptible to ceftazidime and aztreonam (Table 2). Seven different plasmid profiles (b to h) were observed among *E. aerogenes* strains isolated from SCW1, while only four patterns (c, d, g, and h) were observed among those strains recovered from SCW2 (Table 2). Plasmid profile b, consisting of five plasmids ranging from 50 to 10 kb (observed in six isolates), and plasmid profile f, consisting of three plasmids ranging from 60 to 10 kb (observed in one isolate), were unique to SCW1 (Table 2). Two of the three strains isolated from the medical intensive care unit possessed four plasmids ranging from 60 to 10 kb (plasmid profile c), while no plasmids from the other strain were visualized (plasmid profile h) (Table 2). These organisms produced an enzyme resembling SHV-4 and were resistant to ceftazidime, aztreonam, trimethoprim-sulfamethoxazole, and gentamicin. The *E. aerogenes* strains originating from the surgical outpatient clinic had

TABLE 2. Plasmid profiles of *E. aerogenes*

Plasmid profile	No. of plasmids	Approximate size (kb)	Ward (no. of isolates)[a]	Antibiogram results[b,c]				Most likely ESBL
				CAZ	ATM	SXT	GM	
a	5	50, 35, 20, 15, 12	OPC (1)	R	R	R		SHV-4
b	5	50, 45, 35, 20, 10	SCW1 (6)	R	R	R		SHV-4
c	4	60, 45, 20, 10	SCW1 (5)	R	R	R	R	SHV-4
			SCW2 (2)	R	R	R	R	SHV-4
			MICU (2)	R	R	R	R	SHV-4
d	4	45, 35, 20, 10	SCW1 (2)	R	R	R	R	SHV-4
			SCW2 (1)	R	R	R	R	SHV-4
e	3	60, 50, 14	SCW1 (2)	R	R	R	R	SHV-4
			OPC (2)	R	R	R	R	SHV-4
f	3	60, 50, 10	SCW1 (1)	R	R	R		
g	2	50, 10	SCW1 (1)			R	R	SHV-3
			SCW2 (2)	R	R	R	R	SHV-4
			SGW (1)	R	R	R	R	SHV-4
h	0		SCW1 (1)	R	R	R	R	SHV-5
			SCW2 (1)	R	R	R	R	SHV-4
			MICU (1)	R	R	R	R	SHV-4

[a] OPC, outpatient clinic; SCW1, spinal cord injury ward 1; SCW2, spinal cord injury ward 2; MICU, medical intensive care unit; SGW, surgical general ward.
[b] CAZ, ceftazidime; ATM, aztreonam; SXT, trimethoprim-sulfamethoxazole; GM, gentamicin.
[c] R, resistant.

TABLE 3. Characteristics of *Enterobacter* strains and their respective transconjugants

Strain[a]	pI(s) of β-lactamases	Approximate size (kb) of plasmid(s)	Antibiotic inhibition zone diam (mm)[b]				Most likely β-lactamase
			CAZ	ATM	GM	SXT	
E. coli C600N			28	32	20	24	
E. aerogenes 187	8.3, 6.8	50, 10	20	24	12	6	AmpC, SHV-3
E. coli JP01/tr	6.8	50	22	26	14	8	SHV-3
E. aerogenes 200	8.3, 7.8	60, 50, 14	14	12	10	6	AmpC, SHV-4
E. coli JP02/tr	7.8	50, 10	17	15	12	8	SHV-4
E. aerogenes 220	8.3, 7.8	60, 50, 10	15	14	10	6	AmpC, SHV-4
E. coli JP03/tr	7.8	50	17	17	12	8	SHV-4
E. aerogenes 184	8.3, 8.2		15	13	6	6	AmpC, SHV-5
E. coli JP04/tr	8.2		17	16	9	8	SHV-5

[a] *E. aerogenes* 187, 200, 220, and 184 were donors; *E. coli* C600N served as the recipient; and *E. coli* JP01, -2, -3, and -4 were the respective transconjugants.
[b] CAZ, ceftazidime; ATM, aztreonam; GM, gentamicin; SXT, trimethoprim-sulfamethoxazole.

two different antibiograms and plasmid profiles. Two of three isolates, possessing plasmid profile e, were resistant to ceftazidime, aztreonam, trimethoprim-sulfamethoxazole, and gentamicin, while the remaining isolate, with plasmid profile a, appeared to be susceptible to gentamicin (Table 2). All these organisms produced an enzyme resembling SHV-4.

Conjugation experiments. The following strains were selected for conjugation with *E. coli* C600N: *E. aerogenes* 187, producing an enzyme with a pI of 6.8, resembling SHV-3; *E. aerogenes* 200 and *E. aerogenes* 220, producing enzymes with pIs of 7.8, resembling SHV-4; and *E. aerogenes* 184, producing an enzyme with a pI of 8.2, resembling SHV-5. All the strains also possessed an inducible Bush group 1 β-lactamase with a pI of 8.3. A plasmid of approximately 50 kb was transferred from *E. aerogenes* 187, *E. aerogenes* 200, and *E. aerogenes* 220 to *E. coli* C600N (Table 3). No plasmids were visualized in *E. aerogenes* 184 or its transconjugant, *E. coli* JP04/tr (Table 3). IEF performed on the *Enterobacter* strains and their respective transconjugants showed the β-lactamases resembling SHV-3, SHV-4, and SHV-5 present in both donors and recipients. The transfer of plasmids encoding SHV β-lactamase genes into *E. coli* C600N was accompanied by resistance to gentamicin and trimethroprim-sulfamethoxazole and decreased susceptibility to cefotaxime, ceftazidime, and aztreonam (Table 3).

DNA amplification. The strains used in the conjugation experiments were selected for amplification with PCR (as follows): *E. aerogenes* 187 (pI 6.8), *E. aerogenes* 200 (pI 7.8), *E. aerogenes* 220 (pI 7.8), and *E. aerogenes* 184 (pI 8.2) as well as their respective transconjugants, *E. coli* JP01/tr (pI 6.8), *E. coli* JP02/tr (pI 7.8), *E. coli* JP03/tr (pI 7.8), and *E. coli* JP04/tr (pI 8.2). Strains producing SHV-3, SHV-4, SHV-5, and SHV-7 were used as positive controls, while *E. coli* C600N was used as a negative control. A 781-bp fragment specific for SHV β-lactamases was amplified in *E. aerogenes* 187, *E. aerogenes* 200, *E. aerogenes* 220, *E. aerogenes* 184, and their respective transconjugants as well as in the positive controls (Table 3). No amplification was observed with *E. coli* C600N (Table 3). Therefore, the ESBLs produced by these strains are indeed derivatives of an SHV β-lactamase.

DISCUSSION

The prevalence of resistance among *Enterobacter* strains to expanded-spectrum β-lactam antibiotics varies between diverse geographic locations (29). To our knowledge, this is the first study describing a large number of strains of *E. aerogenes* producing different SHV-derived extended-spectrum β-lactamases. The different β-lactamases resembling SHV-3, SHV-4, and SHV-5 as well as resistance to gentamicin and trimethoprim-sulfamethoxazole were transferred into *E. coli* C600N. This was accompanied by a plasmid of approximately 50 kb in some of the strains (Table 3). Although it may seem surprising that an organism with an inducible cephalosporinase would acquire an extended-spectrum β-lactamase, resistance to other agents such as the aminoglycosides and trimethoprim-sulfamethoxazole, which is encoded on the same plasmid as the extended-spectrum β-lactamase, may often be the major factor behind the acquisition of these plasmids by *Enterobacter* (29, 30).

It is important to detect *Enterobacter* strains producing extended-spectrum β-lactamases in a clinical laboratory and to differentiate them from derepressed mutants. Plasmids encoding extended-spectrum β-lactamases may also encode resistance to other classes of antibiotics, such as the aminoglycosides and trimethoprim-sulfamethoxazole, limiting the options of physicians treating infections caused by organisms producing these enzymes (30). Therefore, factors leading to the selection and spread of strains producing ESBLs need to be identified and, where possible, eliminated (29, 30). In this study, strains of *E. aerogenes* producing enzymes resembling SHV-4 and SHV-5 were resistant to ceftazidime, aztreonam, gentamicin, and trimethoprim-sulfamethoxazole, but not necessarily to cefotaxime (Table 1). Thus, for a clinical laboratory to effectively detect species of *Enterobacter* producing extended-spectrum β-lactamases, a combination of cefotaxime with ceftazidime and aztreonam should be included in the test panel for routine susceptibility testing. Strains of *Enterobacter* which are resistant to ceftazidime and aztreonam but which appear susceptible to cefotaxime should be screened for possible extended-spectrum β-lactamase production by a method such as the double-disk potentiation test. This will ensure that the majority of extended-spectrum β-lactamase-producing *Enterobacter* strains will be detected. The detection of these strains is of vital importance, because they can be responsible for the spread of resistance genes in a hospital setting (29). One isolate in this study, *E. aerogenes* 187, producing an enzyme resembling SHV-3, showed decreased susceptibility but not resistance to ceftazidime, cefotaxime, and aztreonam. The detection of these strains not showing frank resistance to the expanded-spectrum cephalosporins or aztreonam remains a challenge for the clinical laboratory.

Enterobacter spp. are important nosocomial pathogens (29). Because of the popularity of the expanded-spectrum cephalosporins, the prevalence of *Enterobacter* spp. will probably continue to increase. Thus, the challenge to clinicians and microbiologists to recognize susceptibility patterns indicative of the presence of specific β-lactamases, such as the extended-spec-

trum β-lactamases, will become even more important as this genus acquires additional antimicrobial resistance mechanisms, as shown in this study.

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